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A Modified 2-Thiobarbituric Acid (TBA) Method for the Determination of Malonaldehyde in Cured Meats

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SUMMARY

A modification of the distillation method of the TBA determination suitable for cured meats is described. Small amounts of nitrite ion are capable of reducing TBA numbers of rancid meat significantly, and the reduction increases linearly with nitrite concentration. This interference takes place during the distillation step in the TBA procedure and is believed to be due to nitrosation of malonaldehyde. Diazonium salt formation with sulfanilamide is utilized to bind the nitrite before beginning the TBA test.

INTRODUCTION

The distillation method for the 2-thiobarbituric acid (TBA) procedure (Tarladgis *et al.*, 1960) has been useful in studies of oxidative rancidity in both plant and animal tissues. Its wide application has prompted interest in the use of this method as a quality control measure by both the cured-meat industry and large purchasers of cured products. It was brought to the attention of these authors that the nitrite present in these cured products interferes in the TBA test (Olson, 1961). Also, observations in this laboratory have indicated that sensory scores and TBA numbers of cured products did not have the high correlation found with uncured cooked meats. For this reason, it was desirable to study the influence of nitrite on the TBA test with hope of modifying the method for cured products.

Hougham and Watts (1958) reported that concentrations of nitrite of 100 ppm or lower did not interfere in the Turner modification of the TBA test. A 20–30% decrease in TBA value was noted when 200 ppm, the legal maximum, was present at the time of analysis. The Turner method involves heating ground meat tissue with TBA in the presence of strong acids, followed by extraction of the red color with a combination of solvents. The method has been criticized because malonaldehyde extraction is incomplete and fat oxidation takes place during the drastic heating necessary for maximum color development (Tarladgis *et al.*, 1960). Since the distillation method differs greatly from the Turner method in procedure and results, the extent of nitrite interference must be reassessed. Also, an attempt can be made to ascertain the location of nitrite interference since the production of malonaldehyde from its precursors and the condensation of malonaldehyde with TBA are separate steps in the distillation procedure.

In searching for a procedure that might prevent nitrite interference with the TBA reaction, oxidation or reduction of nitrite was ruled out because the carbonyl groups of malonaldehyde would also be affected. The reaction of nitrite with certain aromatic amines to give stable compounds appeared to be more promising. It is well known that nitrite diazotizes primary aromatic amines under acidic conditions yielding diazonium salts. These salts may couple with phenols or amines, yielding colored azo compounds. These reactions are useful in the dye industry and in testing for nitrite. Barnes and Folkard (1951) have described conditions under which sulfanilamide is diazotized and *N*-(1-naphthyl)-ethylenediamine is used for coupling.

This paper describes application of diazonium salt formation to controlling nitrite interference in the TBA determination in cured meats. In addition, the influence of nitrite concentration on TBA number is reported.

METHOD

Reagents. *Sulfanilamide reagent.* 0.5% sulfanilamide in 20% HCl (v/v).

Coupling reagent. 0.3% *N*-(1-naphthyl)-ethylenediamine hydrochloride in 1% HCl (v/v). This solution is slightly dark after preparation and becomes more discolored upon storage, even in the dark.

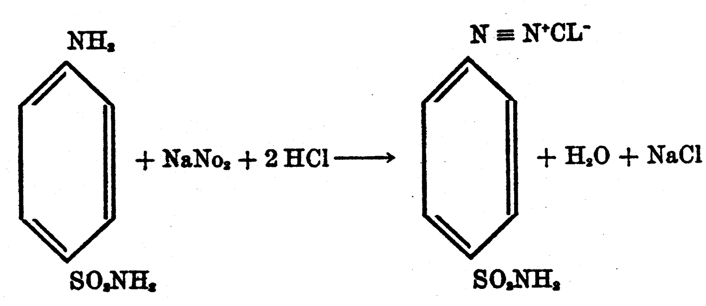
TBA reagent. 0.02*M* 2-thiobarbituric acid in 90% glacial acetic acid.

HCl solution. 1 part of concentrated HCl to 2 parts of distilled water.

Nitrite test (quantitative). The method used is a modification of the Shinn method as proposed by Barnes and Folkard (1951). Sulfanilamide reagent (2 ml) is added to 5 ml of distillate or other sample. After mixing, a reaction period of 5 min is allowed, followed by the addition of 2 ml of the coupling reagent. After another 5 min the optical density at 520 mμ is read against a blank containing 5 ml distilled water plus the testing reagents. Any dilutions must be made to both blank and sample.

A standard curve was prepared, which indicated that the azo dye followed Beer's Law. This standard curve was used to determine the quantity of nitrite present in the distillates.

Nitrite spot test (qualitative). Approximately 0.5 ml of sulfanilamide reagent is placed on nitrite-free filter paper (7 cm) that is pressed onto the meat surface to be tested (ground cooked meats should first be moistened with 10% distilled water). After 5



minutes, 0.5 ml coupling reagent is placed on the filter paper. The presence and intensity of red color are noted. The red color is proportional to the concentration of nitrite.

Procedure for TBA test on cured meats. The method finally adopted for the TBA test in the presence of nitrite follows. A preliminary qualitative nitrite test is required to give the approximate nitrite level. If a mild test is given (less than 100 ppm), blend 10 g meat, 49 ml distilled water, and 1 ml sulfanilamide reagent in a Waring blender for 2 minutes. Transfer the mixture quantitatively into a Kjeldahl flask by washing with 48 ml distilled water. Add 2.0 ml HCl solution to bring pH to 1.5. Carry out remainder of procedure as described by Tarladgis *et al.* (1960).

If a strong nitrite test is given, blend 10 g meat, 48 ml distilled water, and 2 ml sulfanilamide reagent. Transfer with 48.5 ml distilled water and then add 1.5 ml HCl solution.

The need for and the experimental justification for the above recommendations will be clarified by the results presented in the following sections.

RESULTS AND DISCUSSION

The effect of nitrite concentration on TBA number. To determine the extent of nitrite interference, various amounts were added to rancid uncured meat during the blending preceding distillation. The concentrations—25, 50, 100, 150, and 200 ppm—were obtained by adding suitable amounts of a 0.03% sodium nitrite solution to 10 g meat before carrying out the TBA procedure. The TBA numbers and percent interference are in Table 1.

TBA numbers become progressively lower as the concentration of nitrite increases. A linear relationship is obtained when these variables are plotted. These results demonstrate that nitrite interference in the distillation method can be a matter of concern even when low concentrations of the ion are present.

Nitrite tests on the distillates obtained in the preceding experiment indicated that nitrite was distilling over from all samples to which it had been added. Nitrite was not confined to the first or last portions of distillate collected, but was present in each of ten 5-ml portions.

Table 1. Effect of nitrite on TBA number.

Nitrite concentration (ppm)	TBA no.	% interference
0	7.5	....
25	6.9	8
50	5.9	21
100	4.5	40
150	2.9	61
200	1.3	85

An experiment was designed to determine whether the nitrite present in the distillate was interfering in the condensation of malonaldehyde with TBA reagent. Various amounts of sodium nitrite were added to 5-ml portions of a distillate of rancid uncured meat before reaction with TBA reagent. Slight interference was observed only when the sodium nitrite level reached 3×10^{-4} g, the maximum amount legally present in 1 g cured meat, had been added. The natural occurrence of such a large quantity of nitrite in 5 ml of distillate seemed to be highly improbable. A quantitative nitrite determination was made on 5 ml of distillate from a rancid sample of meat to which 200 ppm nitrite had been added before distillation. Less than one-half the amount of nitrite capable of causing interference was present in the distillate from the meat sample. These results clearly indicate that the depressions in TBA number observed in the first experiment were not caused by interference of distillate nitrite with the condensation reaction.

To study the influence of nitrite on the production of malonaldehyde from its precursors, small amounts of it were added to a solution containing 5×10^{-7} moles of 1,1,3,3-tetraethyoxypropane (TEP). This acetal is the standard upon which "TBA number" is based since it yields malonaldehyde upon acid hydrolysis (Sinnhuber and Yu, 1958). The TEP solution was assumed to represent a 10-g sample of meat, and 1, 2, 10, 20, 100, and 200 ppm of nitrite were added on that basis. The results of the TBA test and nitrite determination in the distillate are in Table 2.

As demonstrated by these results, the interference occurs during the distillation step. Even very small amounts (10 ppm) of the NO_2^- ion are capable of producing the effect. It would be expected that this interference would occur to a larger extent in such a model system than in meat, simply because of increased contact of reactants. Also, it is known that nitrite enters into many reactions with meat itself, thereby reducing its effective concentration (Hougham and Watts, 1958). The amount of nitrite in the distillate from the 200-ppm sample provides additional evidence that distillate nitrite is not the source of interference. The presence of nitrite in the distillate may be regarded only as a sign that nitrite interference has taken place during the distillation.

Table 2. Effect of nitrite on production of malonaldehyde from TEP.

Nitrite added (ppm)	TBA no.	Nitrite in distillate (g/5 ml)
0 (5×10^{-7} moles TEP)	2.8	0
1	2.6	0
2	1.9	trace
10	0.1	5×10^{-6}
20	0.0	3×10^{-5}
100	0.0	7×10^{-5}
200	0.0	1.2×10^{-4}

The effect of nitrite may be due to nitrosation of malonaldehyde during distillation. Aliphatic compounds containing carbonyl or other electron-attracting groups adjacent to an active methylene group may undergo nitrosation at this methylene group by nitrous acid or organic nitrites to form oximes (isonitroso compounds). Above 50°C and in the presence of excess nitrosating agent, these oximes may be converted to diketones or ketoaldehydes (Touster, 1953). The structures of the oximes or dicarbonyls that could be formed from malonaldehyde by this process are such that formation of the resonance stable pink pigment with two molecules of TBA (Sinnhuber *et al.*, 1958) would not be possible. Depending upon the amount of nitrosating agent present, all or portions of the malonaldehyde would be rendered unavailable for reaction with TBA, resulting in lowered TBA numbers. Larger quantities of nitrite seem to be required to nitrosate malonaldehyde during its reaction with TBA reagent than during distillation. This may be due to rapid condensation of the malonaldehyde with TBA.

Diazonium salt formation. The results of the preceding experiment point out the need for removing nitrite before the distillation step. The effect of various quantities of sulfanilamide reagent on nitrite interference was determined next. Nitrite is capable of diazotizing this compound to form a diazonium salt at a pH of 1.5 to 1.6 (Barnes and Folkard, 1951), the same pH range required for the production of malonaldehyde from its precursors. Nitrite (200 ppm) was blended with rancid uncured meat, and then 1, 2, 5, and 10 ml of sulfanilamide reagent were added and blended before distillation. To determine the effect of sulfanilamide on the system, 2, 5, and 10 ml of the reagent were added to the same meat without nitrite. The TBA numbers and percent interference appear in Table 3.

Table 3. Effect of sulfanilamide on TBA numbers of plain and nitrited meat.

Nitrite (ppm)	Sulfanilamide (ml)	TBA no.	% interference
0	0	7.5	....
200	0	1.3	85
200	1	5.0	33
200	2	6.7	11
200	5	6.1	19
200	10	5.6	25
0	2	6.2	17
0	5	5.7	24
0	10	5.2	31

These results show that the addition of 2 ml sulfanilamide reagent to nitrited meat reduces interference to a low level generally within the limits of precision of the TBA method. Only traces of nitrite were found in the distillates. The addition of larger amounts increases interference, indicating that sulfanilamide is affecting the TBA procedure. Comparison of TBA numbers of nitrited and plain meat to which the same amount of reagent had been added shows that interference is less when nitrite is present.

The interference due to sulfanilamide can be reduced by using less of this reagent when the sample contains lower concentrations of nitrites. A sample containing 100 ppm nitrite and a control sample without nitrite were treated with 1 ml sulfanilamide reagent. The respective amounts of interference were 9% and 12%. The use of 1 ml of the reagent thus appears to be satisfactory for amounts of nitrite below 100 ppm.

Some cured meats, such as frankfurters and frozen cured pork, were used in experiments similar to the above. In six samples, containing varying amounts of nitrite, the increase in TBA number ranged from 16 to 140%. The modified procedure appears suitable for rancidity testing in cured products.

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